AR201-14074B

III Robust Summaries of Existing Data

Physical Chemical End Point: Partition Coefficient

Test substance:
The study was carried out using the product Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
US EPA OPPTS 830.7570

GLP:
Yes

Year Study Performed:
2002

Remarks about method:
The octanol/water partition coefficient of the test material was determined using High Performance Liquid Chromatography (HPLC).

The HPLC conditions were as follows:
HPLC System: HP 1050
Column: Zorbax SB-C18 (Stable Bond), 250 mm x 4.6 mm ID, 5 micron
Mobile phase: 70:15:15 methanol:THF:pH 2 aqueous buffer (phosphate)
Wavelength: 235 nm
Flow rate: 1ml/min.

THF was used as an additive, because the test substance would not come off the column in any methanol:pH2 buffer ratio.
From preliminary work it was determined that the extinction coefficient of the test substance was considerably less than any of the reference materials. Therefore, the test substance was made up approximately 4 times the concentration (ca. 2,000 mg/L) of the reference compounds (ca. 500 mg/L). Even so, the number of area counts of the test substance was much lower than that of the reference compounds.
The test substance showed 2 closely eluting peaks, with the retention time of the test substance being calculated by averaging the 2 peaks.
Although the reference materials are supposed to bracket the material tested, the table of suggested reference materials taken from OPPTS 830.7570 had no materials with a log Kow greater than 6.2, the test substance had a calculated log Kow of 7.09.

Results:
Value of Log Kow = 7.09

Temperature:
Ambient +/-2˚C

Remarks about Results:
Surface active: Lipophilic
Dissociative: This substance can be dissociative as the HPLC system was run at pH2
Water solubility: Negligible

Conclusions:
Under the conditions of this study, DIACID 1550 had a calculated log Kow of 7.09.

Reliability:
Klimisch data reliability code 1

Remarks on Data Reliability:
Study conducted to GLP and a currently accepted guideline OPPTS 730.7570, which complies with OECD Guideline 117.

Reference:
Doi, J (2002): WESTVACO DIACID 1550, Determination of the octanol/water partition coefficient using HPLC, US EPA OPPTS 830.7570, Study No. 2002-111-005, MeadWestvaco Corporation, USA, AQUA SURVEY, INC. USA

Environmental Fate and Pathway End Point: Biodegradation

Test substance:
The study was carried out using the product Westvaco DIACID® H-240, the potassium salt of Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
40CFR 796.3140

Test Type:
Anaerobic

GLP:
Yes

Year Study Performed:
1992

Contact Time:
56 days

Inoculum:
Anaerobic sludge

Remarks about method:
Inoculum: anaerobic sludge from a treatment plant, concentration of sludge in nutrient medium used was 10% level (100 ml/l)
Concentration of test chemical: 5, 10 and 20 ppm as C, aqueous solution of Westvaco DIACID® H-240 (potassium salt) in nutrient medium
Pre-acclimation conditions: None mentioned
Temperature of incubation: 35˚C (dark)
Dosing procedure: 4 replicates per test level, reference and blank
Sampling frequency: 3, 14, 28, 42 and 55 days from inoculation
Controls and blank system used: ethanol reference and sludge plus nutrient for blank
Analytical method used to measure biodegradation: gas evolution monitoring using a glass manometer
Method of calculating measured concentrations: cumulative arithmetic mean (corrected for blank) and expected theoretical yield

The gas evolution data was confirmed using organic carbon analysis.

Since the product Westvaco DIACID® 1550 is highly insoluble in water this study was conducted using the potassium salt, which has a higher water solubility.

Results:
Degradation value = 84% within 2 months

Conclusions:
Results indicated that the sample degraded up to 84% under anaerobic conditions, based on the interpretation of both gas evolution and residual carbon analysis.

Reliability:
Klimisch data reliability code 1

Remarks on Data Reliability:
The study was conducted according to GLP and conformed to EPA Method 40 CFR 796.3140, which is equivalent to OPPTS Method 835-3400.

The testing was conducted in a large vessel (1 Litre) which allowed for the low concentrations to be measured with reasonable accuracy.

Reference:
Drozdowski D et al (1992): Anaerobic Biodegradability Testing of DIACID 1550 (6339-33) Potassium Salt, Conducted for: Westvaco Chemical Division USA, Test Report No. 064187, United States Testing Company, Inc. Biological Services USA

Environmental Fate and Pathway End Point: Biodegradation

Test substance:
The study was carried out using the product Westvaco DIACID® H-240, the potassium salt of Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
OECD Method 301E

Test Type:
Aerobic

GLP:
Yes

Year Study Performed:
1991

Contact Time:
35 days

Inoculum:
Industrial sewage, adapted

Remarks about method:
Inoculum: mixed culture inoculum: rich top soil, activated sludge from a sewage treatment plant (secondary effluent) and raw surface water, inoculation of test solution with 1 ml/l of the combined inoculum
Concentration of test chemical: 19 ppm as C, aqueous test solution of Westvaco DIACID® H-240 (potassium salt) in nutrient medium,
Pre-acclimation conditions: not required by the modified OECD screening test
Temperature of incubation: 20-25˚C (in the dark, with shaking)
Dosing procedure: inoculation of each flask with 1 ml/l of combined inoculum
Sampling frequency: 0, 7, 14, 21, 28, 35 days after inoculation
Controls and blank system: aniline as reference, nutrient plus sludge as blank
Analytical method used to measure biodegradation: Acidification of supernatant sample purged with nitrogen and analyzed for DOC
Method of calculating measured concentrations: Arithmetic mean, simplified version of the OECD calculation given in method 301E

Since the product Westvaco DIACID® 1550 is highly insoluble in water this study was conducted using the potassium salt, which has a higher water solubility.

Results:
Degradation value = 65% within 28 days

Remarks about Results:
Observed inhibition: non-inhibitory at concentrations of </= 25%
Time required for 10% degradation: 0-7 days (not specified in report), from graphical interpolation 10% degradation requires approximately 2 days
Total degradation at the end of the test: 63.2% degradation after 35 days

Conclusions:
When tested as specified Westvaco DIACID® 1550 potassium salt was not readily biodegradable, showing 65% degradation after 28 days.

Reliability:
Klimisch reliability code 1

Remarks on Data Reliability:
Study conducted to GLP and followed a currently accepted guideline.

Reference:
Drozdowski D et al (1991): Modified OECD Test for Ready Biodegradability, Shake Flask Test of Diacid 1550 Potassium Salt (6339-33), Conducted for: Westvaco Chemical Division, USA, Test Report No. 063576-3B, United States Testing Company, Inc. Biological Services Division USA

Ecotoxicity End Point: Acute Toxicity to Fish

Test substance:
The study was carried out using the product Westvaco DIACID® H-240, the potassium salt of Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
40 CFR Part 797.1400

Test type:
Static

GLP:
Unknown

Year Study Performed:
1991

Species:
Pimephales promelas

Analytical method:
Gas chromatography (Flame Ionization Detector)

Exposure Period:
96 hours

Statistical Method:
Graphical interpolation

Remarks about method:
Parameters about organism:
- age: 28-31 days
- length: </= 18 mm
- weight: not known
- loading: Corrected concentrations: 0, 2.4, 4.9, 9.8, 16.3 and 41.3 ppm
- pretreatment: none, only a screening test to determine the definitive test concentrations

Parameters of Test system:
- Dilution water source: USA EPA moderately-hard reconstituted water
- Dilution water chemistry: hardness - 88 mg/l $CaCO_3$, alkalinity - 74 mg/l $CaCO_3$, pH - 7.7, Conductivity - 232 µmhos, Dissolved oxygen - 8.2 mg/l
- Stock and test solution: stock solution of the analyte Westvaco DIACID® 1550 (potassium salt 39.18% w/v) prepared at a concentration of 1075.1 ppm (activity corrected) in EPA modified hard water. Sequential dilutions were made to produce the test solutions
- Flow-through rate: not applicable as system was static and renewed daily

- Vehicle/solvent and concentrations: diluent in the system was EPA modified hard water, nominal concentrations made were 10.75, 26.75, 53.50, 80.30 and 100.00 ppm of analyte
- Stability of the test chemical solutions: considered to be stable
- Exposure vessel type: 2 L polypropylene test vessels, exposure volume 1 L
- Aeration - mixing test solutions to saturation prior to test if dissolved oxygen falls below 40% during the test oil-free air supplied at 100 +/- 10 bubble per minute
- Lighting - 16:8 hour light/dark cycle fluorescent 50-100 ft candles, 2 vessels per treatment
- Replicates: 2 replicates per treatment, 10 fish per replicate, 20 fish per treatment
- Water chemistry in test: D.O.: control - 8.2 mg/l, 16.3 ppm - 8.0 mg/l, pH: control - 7.7, 16.3 ppm - 7.7
- Test temperature range: 21 +/- 1 ˚C
- Method of calculating mean measured concentrations: arithmetic mean corrected for activity of product

Since the product Westvaco DIACID® 1550 is highly insoluble in water this study was conducted using the potassium salt, which has a higher water solubility.

Results:
Nominal concentration:
10.75, 26.75, 53.50, 80.30 and 100.00 ppm

Measured concentration:
0, 2.4, 4.9, 9.8, 16.3 and 41.3 ppm

Endpoint:
LC50 = 15 ppm (measured) at 96 hours.

Statistical Results:
95% confidence limits not obtainable

Remarks about Results:
Biological observations: Mortality, reflex loss, erratic swim
Table showing cumulative mortality: no effects seen for concentrations 0-9.8 ppm.
For 16.3 ppm the cumulative mortality was as follows: 24 hr- 6, 48 hr- 9, 72 hr- 12, 96 hr- 15 (% mortality = 75)
For 41.3 ppm the cumulative mortality was as follows: 24 hr- 20, 48 hr - 20, 72 hr- 20, 96 hr -20 (% mortality = 100)

Lowest test substance concentration causing 100% mortality: 41.3 ppm only concentration to cause 100% mortality
Mortality of controls: controls were healthy and swam actively
Abnormal responses: none
Reference substances: none used
Any observations, such as precipitation that might cause a difference between measured and nominal values: none recorded

Additional data outside the longest end-point:
24 hr LC50 = 18.5 ppm

48 hr LC50 = 17 ppm
72 hr LC50 = 16 ppm

Conclusions:
The acute toxicity of DIACID 1550 (as potassium salt) to the freshwater minnow, *Pimephales promelas*, was found to be: 96 hour LC50 = 15 ppm (95% confidence limit not obtainable)
The No Observed Effect Concentration (NOEC) was 9.8 ppm

Reliability:
Klimisch data reliability code 2

Remarks on Data Reliability:
The study conformed to EPA Method 40 CFR 797.1400 which is equivalent to OPPTS Method 850.1075. Although there is not a GLP Compliance statement in the report, the test plan (Addendum # 3) indicate that the study was conducted according to GLP.

Reference:
Cooke D (1991): Aquatic Toxicity Tests versus *Pimephales promelas* and *Daphnia pulex* DIACID 1550, Conducted for: Westvaco Chemical Division USA, Test Report No.: 063576-2, United States Testing Company, Inc., Biological Services Division USA.

Ecotoxicity End Point: Acute Toxicity to Daphnia

Test substance:
The study was carried out using the product Westvaco DIACID® H-240, the potassium salt of Westvaco DIACID® 1550

Chemical Category:
Fatty Acid

Method:
40CFR Part 797.1300

Test type:
static

GLP:
Unknown

Year Study Performed:
1991

Species:
Daphnia pulex

Analytical method:
Gas chromatography (Flame Ionization Detector)

Exposure Period:
48 hours

Statistical Method:
Graphical interpolation

Remarks about method:
Test organisms
- USTC stock cultures
- Age at study initiation: </= 48 hours
- Control group: diluent only

Test conditions:
- Stock solutions: vehicle/solvent: EPA moderately-hard reconstituted water, stock solution was prepared at a concentration of 1075.1 ppm (activity corrected). Sequential dilutions were made to achieve nominal concentrations of 10.75, 26.75, 53.50, 80.30 and 100.00 ppm
- Test temperature range: 22-23 ˚C
- Exposure vessel type: test vessel: 18 x 150 mm glass test tubes capped, exposure volume: 15 ml
- Aeration: aerate by mixing test solutions to saturation prior to test no aeration during test, 4 vessels per treatment.

- Dilution water source: EPA moderately-hard reconstituted water
- Dilution water chemistry: hardness: 90 mg/l $CaCO_3$, alkalinity: 80 mg/l $CaCO_3$, pH: 7.7, Conductivity: 233 µmhos, D.O.: 8.4 mg/l
- Lighting: 16:8 hour light/dark cycle, fluorescent, 50-100 ft candles (lab ambient)
- Water chemistry: D.O.: control - 8.4, 16.3 ppm - 8.4, pH: control - 7.7, 16.3 ppm - 7.8
- Endpoints assessed: immobilization
- Test design: 4 replicates per treatment, 5 daphnia per replicate, 5 concentrations (measured) 2.4, 4.9, 9.8, 16.3 and 41.3 ppm
- Method of calculating mean measured concentrations arithmetic mean corrected for activity

Since the product Westvaco DIACID® 1550 is highly insoluble in water this study was conducted using the potassium salt, which has a higher water solubility.

Results:
Nominal concentration:
10.75, 26.75, 53.50, 80.30 and 100.00 ppm

Measured concentration:
2.4, 4.9, 9.8, 16.3 and 41.3 ppm

Endpoint:
LC50 = 22 ppm (measured) at 48 hours.

Statistical Results:
95% confidence limits not obtainable

Remarks about Results:
Biological observations

- Number immobilized as compared to the number exposed: 0/20 for 2.4, 4.9 & 9.8 ppm, 07/20 at 16.3 ppm, 18/20 at 41.3 ppm
- Concentration response with 95% confidence limits: not obtainable
- Cumulative immobilization: 16.3 ppm: 24 hr - 2, 48 hr - 5 (% mortality 25), 41.3 ppm: 24 hr - 7, 48 hr - 18 (% mortality 90)
- Control response: satisfactory

Additional data outside the longest end-point:
24hr LC50 = >41.3 ppm (95% C.L. not obtainable)

Conclusions:
The acute toxicity of Westvaco DIACID® 1550 (as potassium salt) to the water flea, *Daphnia pulex*, was found to be:
48 hour LC50 = 22.5 ppm (95% confidence limit was not obtainable)
The No Observed Effect Concentration (NOEC) was 9.8 ppm

Reliability:
Klimisch data reliability code 2

Remarks on Data Reliability:
The study conformed to EPA Method 40 CFR 797.1300 which is equivalent to OPPTS Method 850.1010. Although there is not a GLP Compliance statement in the report, the test plan (Addendum # 3) indicates that the study was conducted according to GLP.

Reference:
Cooke D (1991): Aquatic Toxicity Tests versus *Pimephales promelas* and *Daphnia pulex* DIACID 1550, Conducted for: Westvaco Chemical Division, USA, Test Report No. 063576-2, United States Testing Company, Inc. Biological Services Division, USA

Ecotoxicity End Point: Acute Toxicity to Algae

Test substance:
The study was carried out using the product Westvaco DIACID® H-240, the potassium salt of Westvaco DIACID® 1550.

Chemical Category:
Fatty Acid

Method:
40CFR Part 797.1050

Test type:
static

GLP:
Yes

Year Study Performed:
1991

Species:
Selenastrum capricornutum

Endpoint:
Growth rate, cell count by hemocytometer

Analytical method:
Total organic carbon analysis (Ionics 1555)

Exposure Period:
96 hours

Statistical Method:
Probit analysis

Remarks about method:
Test organisms
- Laboratory culture: USTC Stock Culture, origin EPA, Cincinnati, Ohio
- Method of cultivation: incubation in appropriate media, at 20 +/- 2 ˚C under 400 +/- 50 ft. candles using a 16:8 light/dark photoperiod, with manual shaking periodically
- Controls: vessels containing algal medium were inoculated and then incubated as for test vessels

Test Conditions
- Test temperature range: 24 +/- 2 ˚C
- Growth/test medium chemistry: pH: 7.4, alkalinity: 30 mg/l $CaCO_3$, hardness: 50 mg/l $CaCO_3$, conductivity: 130µmhos
- Dilution water source: OECD test medium

- Exposure vessel type: 125ml Erlenmeyer Flasks, 50 ml solution volume
- Aeration: mixing test solutions to saturation prior to test no aeration during test, 3 replicates per treatment
- Water chemistry in test: 32 ppm - pH 7.3/7.5 (0/96 hrs), 63 ppm - pH 7.3/7.4 (0/96 hrs), 125 ppm - pH 7.2/7.3 (0/96 hrs), 250 ppm - 7.1/7.2 (0/96 hrs), 500 ppm - 7.1/7.2 (0/96 hrs)
- Stock solutions preparation: 5 g/195 ml test material in test medium, 10,000 and 1,000 ppm, serial dilutions to: 32, 63, 125, 250 and 500 ppm
- Light levels and quality during exposure: continuous light, fluorescent 400-450 ft candles

Test design: 3 replicates per treatment, inoculum density 10,000 cell per ml
Test concentrations: 32, 63, 125, 250 and 500 ppm
Method of calculating mean measured concentrations: Total organic carbon count

Since the product Westvaco DIACID® 1550 is highly insoluble in water this study was conducted using the potassium salt, which has a higher water solubility.

Results:
Nominal concentration:
32, 63, 125, 250, 500 ppm

Measured concentration:
32, 50, 500 ppm confirmed, 63, 125 ppm variable

Endpoint:
EC50-CD = 88 ppm (nominal) at 96 hours
NOEC <= 32 ppm (nominal) based on growth inhibition
LOEC = 40 ppm (nominal) based on growth inhibition.

Statistical Results:
At 96 hours EC point 95% confidence limits upper/lower: EC1- 2.5/39, EC5- 6.7/52, EC10- 11/62, EC15- 16/69, EC50- 55/137, EC85- 112/463, EC90- 126/647, EC95 - 149/1076, EC100- 201/2850

Remarks about Results:
Response of control group satisfactory
Biological observations
- Cell density (per ml of x 10-E4) at each flask at each measuring point: control - 23, 25, 26 (48 hrs) 118, 130, 125 (72 hrs) 180, 194, 200 (96 hrs), 32 ppm- 23, 23, 20 (48 hrs) 95, 97, 80 (72 hrs) 202, 210, 212 (96 hrs), 63 ppm - 22, 19, 18 (48 hrs) 65, 70, 73 (48 hrs) 120, 125, 120 (96 hrs), 125 ppm - 16, 19, 14 (48 hrs) 23, 24, 24 (72 hrs) 45, 37, 38 (96 hrs), 250 ppm 13, 13, 13 (48 hrs) 12, 14, 15 (72 hrs) 20, 19, 18 (96 hrs), 500 ppm < inoculum at each time point
- Growth curves: log plot of cell number vs hrs for each concentration, showed reduced growth at all levels, lower levels produced smaller % inhibition
growth rate inhibition per concentration 48/72/96 hrs: 32 ppm - 12/27/0%, 63 ppm - 20/44/36%, 125 ppm - 36/81/79%, 250 ppm - 48/89/79%, 500 ppm - 100/100/100%
- Observations: Cell growth was insufficient at 24 and 48 hours to establish concentration-effect relationships for all concentrations. Subculturing of cells from

exposure concentrations recovered viable cells, demonstrated by resumption of growth where previously retarded. Test concentrations up to 500 ppm were thus algistatic rather than algicidal. Morphological changes, as smaller cell size were seen at 250 and 500 ppm. It was possible that sample degradation occurred under test conditions, thus exposure to the test material should not be considered to be uniform throughout the 96 hr test period, but only to concentrations established at the onset of testing.

Conclusions:
For Westvaco DIACID® 1550 (as potassium salt) on the basis of 100% active sample.
96 hr EC50 = 87.6 ppm (95% C.L. = 54.5-137.4 ppm)
96 hr EC10 = 39.9 ppm
96 hr EC90 = 192.6 ppm
NOEC approximately 32 ppm.

Test concentrations up to 500 ppm proved that test material was algistatic.

Reliability:
Klimisch data reliability code 1

Remarks on Data Reliability:
The study was conducted according to GLP and conformed to EPA CFR 40 Part 797.1050 which is equivalent to currently accepted OPPTS Method 850.5400 (which follows the general principles of OECD Method 201)

Reference:
Drozdowski D (1991): Algal Acute Toxicity Test of DIACID 1550 Potassium salt (6339-33), Conducted for: Westvaco Chemical Division USA, Test Report No. 063576-3A, United States Testing Company, Inc., Biological Services Division, USA.

Toxicity End Point: Acute Toxicity

Test substance:
The study was carried out using the product Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
No specific guideline is quoted

GLP:
No

Year Study Performed:
1973

Species:
rat

Strain:
Sprague-Dawley

Sex:
Both

Number of males per dose:
2

Number of females per dose:
2

Vehicle:
corn oil, 25% w/v/ suspension

Route of administration:
Oral – by intubation syringe

Remarks about method:
The animals used were classed as 'young'
There were 4 dose groups and animals received a single dose
Concentration administered: 3038; 4556; 6834 and 10250 mg/kg
There was a 14 day post dose observation period

End Point:
Acute lethal value = 6176 mg/kg bw

Deaths per dose:

Deaths occurred in 0/4, 0/4, 3/4 and 4/4 rats treated at 3038, 4556, 6834 & 10250 mg/kg respectively

Remarks about Results:
Rats dosed at 6834 mg/kg died between 6-22 hours and 2 days after dosing; 1 female rat 6-22 hours after dosing and 1 male and 1 female rat died 2 days after dosing.
Rats dosed at 10250 mg/kg died between 6-22 hours and 2-3 days after dosing; both females died 6-22 hours after dosing and 1 male died 2 days after dosing and the remaining male died 3 days after dosing.

Clinical signs at 3038 mg/kg: hypoactivity and ruffed fur which onset 1 hour after dosing with a duration of 1 day.
Clinical signs at 4556 mg/kg: hypoactivity and ruffed fur which onset 1 hour after dosing with a duration of 2 days, laboured breathing onset after 3 hours with a duration of 6-22 hours.
Clinical signs at 6834 mg/kg: hypoactivity and ruffed fur which onset 1 hour after dosing with a duration of 5 days, laboured breathing onset after 2 hours with a duration of 2 days. Muscular weakness onset 3 hours after dosing with a duration of 2 days. Diuresis onset 6-22 hours after dosing with a duration of 2 days.
Clinical signs at 10250 mg/kg: hypoactivity and ruffed fur which onset 1 hour after dosing and laboured breathing and muscular weakness onset after 2 hours Diuresis onset 6-22 hours after dosing with a duration for all signs until death.

All animals that died underwent necropsy examination and revealed gastroenteritis. No gross pathologic alterations were noted among the animals sacrificed at the end of the 14 day observation period.

Conclusions:
The LD50 is 6176 mg/kg bw for acute oral toxicity to rats.

Reliability:
Klimisch data reliability code 2

Remarks on Data Reliability:
The study was not conducted according to any recognised guideline and only 4 animals per dose group were administered the compound. The observation period was appropriate for an acute study and generally recognised procedures appear to have been followed. Although the study does not comply with any acceptable guidelines (past or current) in the interests of animal welfare, these results should be considered acceptable.

Reference:
Hintz C *et al*. (1973): Report to Westvaco: Acute toxicity studies with DIACID 1550, P.O. No. S-10590, IBT No. 601-04128, Industrial BIO-TEST Laboratories, Inc.

Toxicity End Point: Bacterial Gene Mutation

Test substance:
The study was carried out using the product Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
Method used followed all procedures outlined by Ames – Ames test

GLP:
Yes

Year Study Performed:
1991

Species:
Salmonella typhimurium Strains used were TA 98, 100, 1535, 1537 and 1538

Metabolic Activation:
activator mammalian liver S-9, with or without

Concentration:
10 mg/ml, 100 mg/ml in Dimethylsulfoxide

Statistical Method:
The average number of revertant colonies (+/- SD)

Remarks about method:
Test Design
- Number of replicates: 2 plates for 10 mg/ml and 3 plates for 100 mg/ml, plus 1 solvent and 1 positive control, with activation and the same without activation per strain
- Frequency of Dosing: one dosing only, followed by a 48 hour incubation period
- Positive and negative control groups and treatment: 1 positive and 1 negative control group and two treatment groups with and without activation per strain

Solvent/vehicle: solvent used was dimethylsulfoxide a concentration of 10 and 100 mg/ml of test material

Criteria for evaluating results: spontaneous reversion frequency is measured. A test material producing a consistent bacterial response twice that of the solvent or spontaneous reversion count is indicated as positive for TA 98 and 100, a three times response is indicated as positive for TA 1535, 1537 and 1538

Results:
Negative

Cytotoxic concentration:

100 mg/l

Genotoxic Effects:
Unconfirmed

Statistical Results:
None, average (+/- SD) revertant colony counts for all strains at both concentrations and with or without activation were comparable to the solvent control in all cases.

Remarks about Results:
The test material had a maximum solubility in dimethylsulfoxide of 100 mg/ml and is sparingly soluble in water

Conclusions:
When tested as specified Westvaco DIACID® 1550 did not exhibit mutagenicity versus test strains TA 98, 100, 1535, 1537 and 1538 in the presence or absence of activation at maximum solubility and cytotoxicity.

Reliability:
Klimisch reliability code 1

Remarks on Data Reliability:
The test was not carried out according to currently accepted guidelines, but it was conducted to GLP and followed a method which is still referenced in current guidelines.

References:
Tong C C (1991): Ames Mutagenicity Testing of DIACID 1550, Conducted for: Westvaco Chemical Division, USA, Test Report No. 063576-1B, United States Testing Company, Inc, Biological Services Division, USA,

Ames et al (1975): Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsome Mutagenicity test, Mutation Research, 31, 347-364

Toxicity End Point: In vitro gene Mutation

Test substance:
The study was carried out using the product Westvaco DIACID® 1550

Chemical Category:
Fatty acid

Method:
OECD 473 Chromosomal aberration in cultured Chinese hamster ovary cells

GLP:
Yes

Year Study Performed:
1991

Species:
Chinese Hamster Ovary cells

Metabolic Activation:
S-9 activation system 7.5 ml/100 ml (S-9 to culture medium)

Concentration:
non-activated: 13, 25, 50, 75, 100, 150 µg/ml, activated: 6.3, 13, 25, 50, 75, 100 µg/ml

Statistical Method:
Chi-square test for positive controls only

Remarks about method:
Test Design

- Number of replicates: 4 per dose level, 2 for cytotoxicity test and 2 for chromosome aberration assay
- Frequency of Dosing: 1 dose, exposure to test material for 2 hours with activation and 16 hours without activation followed in both cases by incubation and additional harvesting time
- Positive controls - triethylenemelamine (0.5 µg/ml) non-activation and cyclophosphamide (30 µg/ml) activation
- Number of metaphases analyzed for chromosomal studies: 100 metaphases at each dose level (50 per duplicate group). Only cells showing 18-20 chromosomes were scored.

Solvent used was dimethylsulfoxide (DMSO), at a maximum of 1% of the volume of the culture medium
This main study was preceded by a range-finding study with concentrations from 0.1-5000 µg/ml (10 levels)
Number of metaphases analyzed for chromosomal studies: 100 metaphases at each dose level (50 per duplicate group). Only cells showing 18-20 chromosomes were scored

Results:
Negative

Cytotoxic Concentration:
At 100 µg/ml the Relative Cell Growth (RCG) was reduced to 54% (non-activated) and 13% (activated)

Genotoxic Effects:
Unconfirmed

Statistical results:
Chi-square test was used on positive controls, results were considered significant if p is </= 0.05. There were no statistically significant results in the test doses.

Remarks about Results:
Miscibility was tested during the range-finding test and indicated the test article formed a turbid suspension with water. There was precipitate in the medium in the test flasks at concentrations of 500, 1000 and 5000 µg/ml in DMSO, indicating miscibility limitations at this level. However, since cells did not survive at these doses, thus these levels were not used in the main study. Osmolality values were determined during the range-finding test, again at levels 500, 1000 and 5000 µg/ml precipitate was visible in the medium. However, none of the doses tested showed an osmolality beyond 427 (mOsmol/kg water) which was below the range that may cause damage to the cells.

There were no dose-related effects seen. In the non-activated system there were no scorable metaphases at 150 µg/ml, chromosome aberrations were scored from controls and 25, 50, 75 and 100 µg/ml. In the activated system there were insufficient scorable metaphases at 100 µg/ml, chromosome aberrations were scored from 13, 25, 50 and 75 µg/ml.

No. of aberrations per cell (non-activated) 0.01-0.03 for 25-100 µg/ml, </= to solvent values.
No. of aberrations per cell (activated) 0.01-0.02 for 13-75 µg/ml, < solvent values

Conclusions:
Under the conditions of this study, Westvaco DIACID® 1550 did not induce a significant increase in chromosome aberrations nor was there any indication of a positive dose trend in either the non-activated or activated systems. Therefore the test article is considered to be negative in the in-vitro CHO chromosome aberration assay.

Reliability:
Klimisch reliability code 1

Remarks on Data Reliability:
Study conducted to GLP and accepted guideline.

Reference:

Kumaroo, P V (1991): Test for Chemical Induction of Chromosome Aberration in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation, Westvaco Chemical Division, USA, Study No. 0172-3110, Sitek Research Laboratories, USA